


The number of previous failed embryo transfer cycles is an independent factor affecting implantation rate in women undergoing IVF/ICSI treatment

A retrospective cohort study

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Abstract

The implantation rate (IR) in assisted reproductive technologies such as in vitro fertilization (IVF) and intracytoplasmic sperm injection is affected by many different factors such as age, quality of embryo, and stage of embryo development. This study aimed to investigate to what extent the number of previous failed embryo transfer cycles is an independent factor affecting IR.

This was a single-center, retrospective cohort study of a consecutive series of 6376 day-3 embryo transfer (ET) cycles following IVF between January 2012 and August 2018. None of the subjects underwent endometrial scratch/injury prior to the treatment cycle, or received intravenous immunoglobulin, steroid, dehydroepiandrosterone, intralipid or heparin during the treatment with the aim of improving implantation rates.

Multiple regression analysis showed that the 3 most important independent factors affecting the IR, in decreasing of importance: age, frozen or fresh embryo transfer and the number of previous ET cycles. Having controlled for 2 of the more important confounding variables including maternal age and the type of embryo, the IR in women who had 0, 1, 2, and 3 or more previous failed ET cycles were 45.8%, 35.9%, 31.2%, 21.0%, respectively ($P < .001$).

Repeated implantation failure is a significant independent factor affecting the IR. The number of previous failed ET cycles should be considered in counselling women regarding the prognosis of a further IVF-ET treatment cycle.

Abbreviations: ET = embryo transfer, ICSI = intracytoplasmic sperm injection, IR = implantation rate, IVF = in vitro fertilization, PR = pregnancy rate.

Keywords: embryo implantation, embryo transfer, in vitro fertilization, intracytoplasmic sperm injection, pregnancy outcome

1. Introduction

In women with recurrent miscarriage, a commonly asked question is whether the number of previous miscarriages is a significant prognostic factor for a subsequent pregnancy. Some studies have reported that the prognosis is unaffected for up to 3 miscarriages.^[1–4] One possible explanation is that the

likelihood of miscarriage in each pregnancy is primarily an independent event, with embryo factors such as aneuploidy being significant but randomly occurring events. However, other studies have reported that the higher the number of previous miscarriages, the more likely it is miscarriage will occur in a subsequent pregnancy.^[5–8]

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The same question may be asked of women experiencing repeated failure of embryo transfer (ET) following in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Although the results of IVF/ICSI-ET have been steadily increasing over recent years, with an overall clinical pregnancy rate of 40% per treatment cycle reported in 2017 to 2018,^[9–11] it still means that 60% of women are unable to conceive successfully with each treatment cycle. It also follows that 20% of women will fail to conceive after 3 IVF/ICSI attempts. Women who contemplate having further treatment cycle often ask if the chance of success with a further treatment cycle is reduced significantly. Contrary to what most patients and doctors expect, several studies reported that the clinical pregnancy rate in women who have had repeated IVF failure was not significantly reduced compared with the first or second treatment cycles.^[12–15] For example, French National IVF Registry^[14] reported that the pregnancy rate of IVF in the first, second, third, fourth, and fifth ET cycle and in additional subsequent ET cycles were 18.3%, 17.1%, 17.8%, 16.8%, 16.2% respectively. However, there are also a number of studies reporting that the clinical pregnancy rate in women with repeated ET failure is reduced.^[16–19]

There are several possible explanations for the controversial observations. First, women who have had repeated ET failure may or may not be treated differently from women who have their first or second IVF treatment. One notable example is the number of embryos replaced. Women with repeated such treatment failure are more likely to have a greater number of embryos replaced. Second, such women can undergo further investigation and receive additional treatment prior to embarking on in further cycles. For example, some infertility specialists now recommend endometrial scratch or injury immediately prior to commencement of IVF/ICSI treatment^[20–22] with a view to improving the implantation rate. Several randomized controlled trials and meta analyses have shown that endometrial scratch in women with recurrent implantation failure is likely of benefit for implantation.^[23–25] In addition, it is also quite likely that assisted hatching or blastocyst transfer are more likely to be used in women with repeated IVF/ICSI failure.^[26–28]

Given that practice in different infertility centers often vary significantly, it is difficult to establish to what extent the outcome of treatment is affected by a history of repeated failure. The situation is likely to be complicated by the increasing use of various empirical therapies, such as intravenous immunoglobulin,^[29,30] steroid,^[31] heparin,^[32] and dehydroepiandrosterone.^[33]

Here, we conducted a single-center, retrospective cohort study, aiming to identify factors that might have affected the outcome of ET in a group of women who had day-3 embryo transfer, with special reference to the impact of a history of a previous ET failure and the extent to which it might affect the outcomes, after controlling for significant confounding variables.

2. Methods

2.1. Subjects

This was a single-center, retrospective cohort study of a consecutive series of 6376 patients undergoing IVF or ICSI treatment in an assisted reproductive technology unit in China between January 2012 and August 2018. If a couple had undergone more than 1 treatment cycle, only the last cycle was

included for analyze. During the period of study, 94.5% of ET cycles used day-3 embryos, with the remaining 5.5% of ET cycles used blastocyst. For the purpose of this study, only ET cycles of day-3 embryo were included. None of the subjects underwent hysteroscopy or endometrial scratching/injury, or received intravenous immunoglobulin, steroids, dehydroepiandrosterone, intralipid, or heparin immediately prior to the index treatment cycle.

2.2. Protocol for ovarian stimulation

The protocol for ovarian stimulation has been previously described.^[34] In summary, two ovarian stimulation protocol, long gonadotropin-releasing hormone agonist protocol and short gonadotropin-releasing hormone agonist protocol were used. Recombinant follicle-stimulating hormone was used throughout the study to stimulate oogenesis and luteal phase support consisted of intramuscular injections of progesterone in oil at 40 mg/d for one day, beginning from the night of oocyte retrieval and then at 80 mg/d for at least 2 weeks.

2.3. Preparation protocols for frozen-thawed ET

For women with regular menstrual cycles, the frozen-thawed embryos were replaced in spontaneous cycles. For women with irregular or anovulatory cycles, the frozen-thawed embryos were replaced in hormone replacement cycles. The details of these 2 types of cycles used in our center has previously been described.^[35]

2.4. Assessment of embryo quality

Embryo freezing or fresh transfers were performed on the third day after oocyte retrieval. Embryo quality assessment was based on morphology and rate of development in culture. Four grades of embryos were defined.^[36] Embryos with a normal cleavage rate (6–8 cells on the third day) and <20% fragmentation were defined as “good quality.”

2.5. Embryo culture, freezing and thawing

GIII series sequential media^[37] was used for embryo culture. Fresh ET and embryo freezing were performed on the third day after ovum pick-up. Embryos were cryopreserved and thawed using Testart’s standard protocol.^[38] Embryos were held at -7°C for manual seeding and thawed in a 30°C water bath. A programmable freezer (Planner, Middlesex, UK), and embryo freezing and thawing kits (Irvine Scientific, Santa Ana, CA) were used.

2.6. Definition of outcome

Biochemical pregnancy was confirmed by detecting an increased serum β -human chorionic gonadotropin (β -hCG) concentration 12 days after ET. Clinical pregnancy was defined by the observation of a gestational sac with or without a fetal heartbeat on ultrasound evaluation on the 35th day after ET. The number of sacs was taken to be the number of successful implantation. In this study, both clinical pregnancy rate (PR) and embryo implantation rate (IR) were analyzed, but the IR was considered as the primary outcome because the PR is affected by the number of embryos transferred. The clinical PR was defined as the ratio of

the number of cycles with visible gestational sac on ultrasound evaluation (with or without a heartbeat) to the total number of embryo transfer cycles. The biochemical PR was defined as the ratio of the number of cycles with a transient elevation of serum hCG levels without the observation of gestational sac by ultrasound scanning to the total number of transfer cycles. The implantation rate was defined as the total number of visible gestational sacs on ultrasound evaluation to the total number of embryos replaced. A previous IVF/ICSI failure was defined as unable to achieve a live birth with each embryo transfer cycle.

2.7. Matching of sub-groups

Multiply regression stepwise analysis was used initially to identify independent variables affecting the IR. The possible impact of previously failed ET cycles was further examined after important confounding variables such as the woman's age, and the type of embryo used for transfer (fresh or frozen) were sequentially controlled and matched in the sub-groups. Age matching was carried out according to the following steps: 1. stratify age into different bands (24–30, 30–35 and <40 years; women over 40 years were excluded); 2. compare the proportion of subjects in each age band of the sub-groups; 3. determine the number of subjects which needed to be removed to achieve equality of the proportion in each age band of the subgroups; 4. use random number table to identify subjects to be (randomly) excluded. Similar steps were taken to match for the proportion of fresh to frozen embryo transferred in each sub-groups.

2.8. Statistical analysis

All statistical analyses were performed with the use of SPSS 16.0 (SPSS Inc., Chicago, IL). Comparison between groups was made by using one-way analysis of variance for continuous variables and Chi-square for categorical variables, as appropriate. A *P* value of <.05 was considered statistically significant.

2.9. Ethics

The study was approved by the Reproductive Medical Ethics Committee of Sir Run Run Shaw Hospital, affiliated to Zhejiang University, Hangzhou, China (reference SRRSHRMEC) on April 6, 2019, as a service evaluation project as it was a retrospective study with no extra intervention required.

3. Results

3.1. Patients characteristics

A total of 6376 consecutive day 3 embryo transfer cycles following IVF/ICSI between January 2012 and August 2018 were included in the study. Overall, the mean \pm SD age of the women was 31.2 ± 5.0 years. The underlying causes of infertility were: tubal factor, 49%; male factor, 23%; anovulatory, 6.8%; endometriosis, 5.2%; and unexplained infertility, 16%. Among the ET cycles, 49.2% were fresh and 50.8% were frozen-thawed embryo transfers.

3.2. Factors affecting implantation

In Table 1, the various possible factors affecting implantation were compared between those who did and did not have implantation in the ET cycle. Successful implantation was found

Table 1

Factors affecting implantation.

Variables	Implanted	Failed to implant
Age (yr) [‡]	31.0 \pm 3.9	32.5 \pm 4.6
Proportion of fresh ET cycles (%) [‡]	42.1	54.0
No. of failed ET cycles [‡]	0.3 \pm 0.6	0.4 \pm 0.7
BMI	22.0 \pm 0.7	21.0 \pm 0.5
infertility years	2.0 \pm 0.5	2.0 \pm 0.7
the number of embryos transferred per ET cycle	2.2 \pm 0.7	2.0 \pm 0.5
etiology of infertility	NS	NS
ovarian stimulation protocol	NS	NS
endometrial thickness	8.0 \pm 0.5	7.9 \pm 0.5

BMI = body mass index, ET = embryo transfer.

[‡] *p* < .001, proportion was compared with the use of Chi-square test.

NS = not significant.

data were present as mean \pm SD.

to be associated with the following factors: Age, the type of embryo transfers (frozen or fresh) and the number of previous ET cycles.

3.3. Multiple regression analysis

We first performed multiple regression analysis using the IR as the dependent variable, and those factors identified in Table 1 which appear to affect the IR rate as independent variables: age, body mass index, etiology of infertility, infertility years, ovarian stimulation protocol, endometrial thickness, the number of embryos transferred per ET cycle, number of previous ET cycles, and the use of fresh or frozen ET.

Using stepwise analysis, 3 variables were selected in the following order: age, fresh or frozen ET, number of previous ET cycles. The impact of these variables on the IR is shown in Table 2. Maternal age was the most important factor affecting implantation rate, which dropped steadily and significantly from 47.4% in women <30 years, to 16.7% in women \geq 40 years. The use of frozen-thawed embryos for transfer was the second most important factor, with an IR of 46.1%, significantly higher than the implantation rate of 34.6% observed in fresh transfers.

Table 2

The impact of various independent variables affecting IR on the IVF/ICSI-ET treatment outcome.

Variables	No. of cycles	PR (%)	IR (%)
Age (yr) ^{†,‡}			
<30	2161	68.4	47.4
30 \leq age <35	2603	62.0	41.6
35 \leq age <40	1273	49.3	31.4
\geq 40	339	27.3	16.7
Fresh or frozen ET ^{†,‡}			
Fresh ET	3136	52.3	34.6
Frozen ET	3240	67.5	46.1
No. of failed ET cycles ^{†,‡}			
0	4472	61.8	43.1
1	1415	59.4	37.4
2	345	53.7	31.5
3	144	42.6	25.2

ET = embryo transfer, ICSI = intracytoplasmic sperm injection, IR = implantation rate, IVF = in vitro fertilization.

[†] *P* < .001, comparison of pregnancy rate (PR) with the use of Chi-square test.

[‡] *P* < .001, comparison of implantation rate (IR) with the use of Chi-square test.

Table 3

The impact of the number of failed ET cycles on embryo implantation rate and clinical pregnancy rates following matching for age and the proportion of fresh/frozen ET cycles.

Group	0	I	II	III	p value
No. of ET cycles	1079	475	136	85	
Age [†]	33.0±3.8	33.0±3.7	33.1±3.9	33.4±4.0	NS
Proportion of fresh/frozen ET (%)	27.0	27.0	27.5	26.9	NS
No. of embryos transferred in index cycle [‡]	2.0±0.5	2.1±0.7	2.2±0.7	2.1±0.7	<.001
Clinical pregnancy rate/ET (%)	65.5	55.0	53.3	34.5	<.001
Embryo implantation rate/ET (%)	45.8	35.9	31.2	21.0	<.001

ET = embryo transfer.

[†] results are presented as mean ± SD.

NS = not significant; ET = embryo transfer.

Group: 0 = no ET cycle; I = one ET cycle; II = two ET cycles; III = three or more ET cycles.

3.4. Impact of the number of previous failed ET cycles

The third most important variable was the number of previously failed ET cycles. The IR steadily dropped from 43.1% in women with no previous transfer to 25.2% in women who had had 3 failed ET attempts. Table 3 shows a more in-depth analysis of the impact of failed ET cycles on the IR, independent of the effect of maternal age and the use of frozen-thawed embryos, after controlling for the latter 2 variables. The IR in women who had 0, 1, 2, and 3 or more ET cycles showed a steady decline from 45.8% in women with no previous transfers to 21.0% in women who had ≥3 failed transfers ($P < .001$).

4. Discussion

Here, we analyzed to what extent is the number of previous failed embryo transfer cycles an independent factor affecting implantation rate in women undergoing IVF/ICSI treatment. Several factors were found to be significant, in decreasing order of importance: age, frozen or fresh embryo transfer, and number of previous ET cycles. Using multiple regression analysis maternal age was found to be the most important variable. This is not surprising as it has been consistently found to be 1 of the most important factors affecting IVF/ICSI outcomes.^[39] In our study, we found that the IR in women <30 years was 2.8 times that of women ≥40 years.

The second most important variable affecting the IR was the use of frozen-thawed embryos, with an IR (46.1%) significantly ($P < .001$) higher than that of fresh embryos (34.6%). It is of interest to note that several recent studies have suggested that the IR in frozen embryo transfer cycle was significantly higher than that in fresh cycle^[40–42] because endometrial function is compromised in cycles hyperstimulated with estradiol or progesterone, especially if there is elevation of progesterone on the day of hCG administration.^[43,44]

After age and the use of frozen or fresh embryo for transfer, the third most important factor appeared to be the number of previous failed ET cycles. The uncontrolled data in Table 2 showed that the IR in women who had 0, 1, 2, ≥3 failed ET cycles were 43.1%, 37.4%, 31.5% and 25.2% respectively. This suggests that the IR in women with ≥3 failed ET cycles was reduced by 17.9%, compared with those who did not have any transfer. However, having controlled for the more important variables (age of women and types of embryo transferred), we found that the IR in women who have had 0, 1, 2 or ≥3 failed ET cycles were 45.8%, 35.9%, 31.2% and 21.0% respectively. In this situation, the IR in women with ≥3 failed ET cycles was

reduced even further, by 24.8%, when compared with those who did not have any transfer failure. In another words, the IR in women with no previous transfers was more than twice that of women with ≥3 failed transfers. The results show convincingly that the number of previously failed ET cycles has a significant impact on IR, independent of the effect of age of the women and the use of fresh or frozen embryo for the transfer.

Given that practices often vary significantly among different infertility centers, the strength of this study is that a large number of cycles were carried out in a single reproductive center. Because we had applied endometrial scratch and heparin therapy to women with RIF in recent 2 years, thus we intentionally included data before 2018. The limitation of this study was its retrospective nature, and the inclusion of only day-3 embryo transfers, and thus these findings might not apply to blastocyst transfers.

Here we found that IR in women undergoing IVF treatment was affected by a number of independent variables, including, in decreasing order of importance, age of women, the type of embryos (frozen or fresh) used, and the number of failed ET cycles.

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